ANTIMICROBIAL EFFECT OF BEE HONEY IN COMPARISON TO ANTIBIOTICS ON ORGANISMS ISOLATED FROM INFECTED BURNS

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SUMMARY. Despite recent advances in antimicrobial chemotherapy and burn wound management, infection continues to be an important problem in burns. Honey is the most famous rediscovered remedy that is used to treat infected wounds and promote healing. The present study aims to evaluate the antimicrobial effect of bee honey on organisms isolated from infected burns in comparison to the antibiotics used in treatment of burn infection, and to evaluate the effects produced when bee honey is added to antibiotic discs. Thirty patients with burn infection were selected for this study. The collected specimens were cultured on blood agar plates. The isolated colonies were identified by different methods. The isolated organisms were inoculated onto Müller-Hinton agar. Each agar plate was divided by a marker pen into two halves - in one half the antibiotic discs were plated while on the opposite side each antibiotic disc, immersed in honey, was plated opposite to the same antibiotic disc. At the centre of the agar, a sterile filter paper disc immersed in honey was applied. The most frequently isolated organism was *Pseudomonas aeruginosa*, representing 53.3% of the isolates. The mean inhibition zones (in mm) produced by honey (18.2 ± 2.5 mm) when applied to isolated gram-negative bacteria were significantly higher than amoxicillin/clavulinic acid, sulbactam/ampicillin, and ceftriaxone ($p_1 = 0.005$ for each). When honey was added to the antibiotic discs there was highly significant increased sensitivity of isolated gram-negative bacteria compared with each of the antibiotic discs alone and with honey alone. The susceptibility of isolated staphylococci revealed the synergistic effect of added honey to the antibiotic discs tested. The antimicrobial effect of honey (18.7 ± 2.2 mm) was significantly higher than antibiotics - ciprofloxacin, sulbactam/ampicillin, ceftriaxone, and vancomycin ($p_1 \leq 0.05$ for each). After the addition of honey to the tested antibiotic discs there were highly significant increased inhibition zones of antibiotic mixed with honey compared with antibiotic alone - ciprofloxacin, vancomycin, and methicillin ($p_3 \leq 0.001$ for each). Also, the increase was significant compared with antibiotics alone - imipenem, amoxicillin/clavulinic acid, and ceftriaxone ($p_3 \leq 0.05$). In conclusion, honey had more inhibitory effect (85.7%) on isolated gram-negative bacteria (*Pseudomonas aeruginosa, Enterobacter* spp., *Klebsiella*) than commonly used antibiotics, while it had an inhibitory effect on all methicillin-resistant *Staphylococcus aureus* (100%) compared with antibiotics used. A synergistic effect of honey was observed when it was added to antibiotics for gram-negative bacteria and also for coagulase-positive staphylococci.

Introduction

Infection is the commonest and most serious complication of burn injury. Sepsis accounts for 50-60% of deaths in burn patients despite improvements in antimicrobial therapy. The burn wound is an ideal substrate for bacterial growth and provides a wide portal for microbial invasion from the surrounding skin and the burn unit environment.[1] The present study aims to evaluate the antimicrobial effect of bee honey on organisms isolated from infected burn wounds compared with that of certain antibiotics that are commonly used in the treatment of infected burns.

Subjects and methods

Thirty patients admitted to the Mansoura Emergency Hospital burns unit in Egypt developed burn wound infection 2-3 days after admission to hospital. The specimens collected from the wounds were cultured on blood agar plates.

Nine antibiotic discs were selected (Oxoid). Five were used for both gram-negative and gram-positive bacteria, including imipenem (IPM), ciprofloxacin (CIP), amoxicillin/clavulinic acid (AMC), sulbactam/ampicillin (SAM), and ceftriaxone (CRO). Two antibiotic discs specific for gram-negative bacteria were used: amikacin (AK) and aztreonam (ATM). The discs used for gram-positive bacteria were vancomycin (VA) and methicillin (ME).

Honey discs were prepared by using dry sterile filter papers (having the same thickness and size, 6 mm, as the antibiotic discs) immersed in citrus bee honey.

The isolated organisms were adjusted and inoculated onto Müller-Hinton agar. Each agar plate was divided by a marker pen into two halves - the antibiotic discs were plated in one half and, on the opposite side, each antibiotic disc immersed in honey was plated opposite the same antibiotic disc. At the centre of the agar, a sterile filter paper disc immersed in honey was applied (*Fig. 1*).

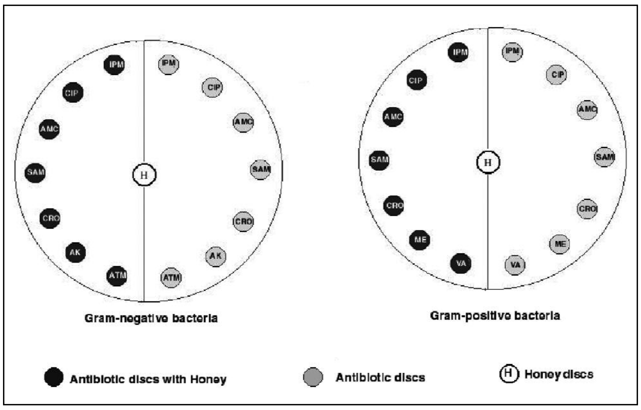

Fig. 1 - The discs used.

Cultured plates were incubated at 37 ºC for 24 h. The diameter of clear inhibition zones around the antibiotics was evaluated by National Committee for Clinical Laboratory Standards.[2]

Results

Cultures of infected burns revealed that *Pseudomonas aeruginosa* was the most frequently isolated organism, representing 53.3% of the isolates, followed by coagulase-positive staphylococci (30%) and then *Enterobacter* spp. 10%); the least frequently isolated was *Klebsiella* (6.7%).

Table I and *Figs. 2* and *3* demonstrate the effect of antibiotics, honey, and a combination of both on isolated gram-negative organisms. The isolated gram-negative

Table I - Effect of antibiotics, bee honey, and a combination of both on isolated gram-negative bacilli (no. = 21)

	Antibiotic		Bee honey		Antibiotic + bee honey		p		
	No. (%)	Inhibition zone in cm (mean ± S.D)	No. (%)	Inhibition zone in cm (mean ± S.D)	No. (%)	Inhibition zone in cm (mean ± S.D)	p_1	p_2	p_3
IPM	18 (85.7%)	18.2 ± 4.2	18 (85.7%)	18.2 ± 2.5	21 (100%)	21.9 ± 4.2	>0.05	<0.001	0.005
CIP	6 (28.6%)	18.0 ± 1.7			18 (85.7%)	20.7 ± 2.6	>0.05	0.005	<0.001
AMC	6 (28.6%)	16.2 ± 0.9			19 (90.5%)	19.9 ± 2.9	0.005	0.025	<0.001
SAM	8 (38.1%)	15.1 ± 2.9			18 (85.7%)	20.0 ± 3.6	0.005	0.05	<0.001
CRO	8 (38.1%)	15.5 ± 1.8			20 (95.2%)	19.8 ± 2.9	0.005	0.025	<0.001
AK	21 (100%)	18.1 ± 4.3			21 (100%)	22.6 ± 4.8	>0.05	<0.001	<0.001
ATM	12 (57.1%)	18.6 –± 3.1			19 (90.5%)	21.9 ± 3.5	>0.05	<0.001	0.005

p_1 = bee honey versus antibiotic
p_2 = antibiotic mixed with bee honey versus bee honey alone
p_3 = antibiotic mixed with bee honey versus antibiotic alone
p is significant if ≤ 0.05

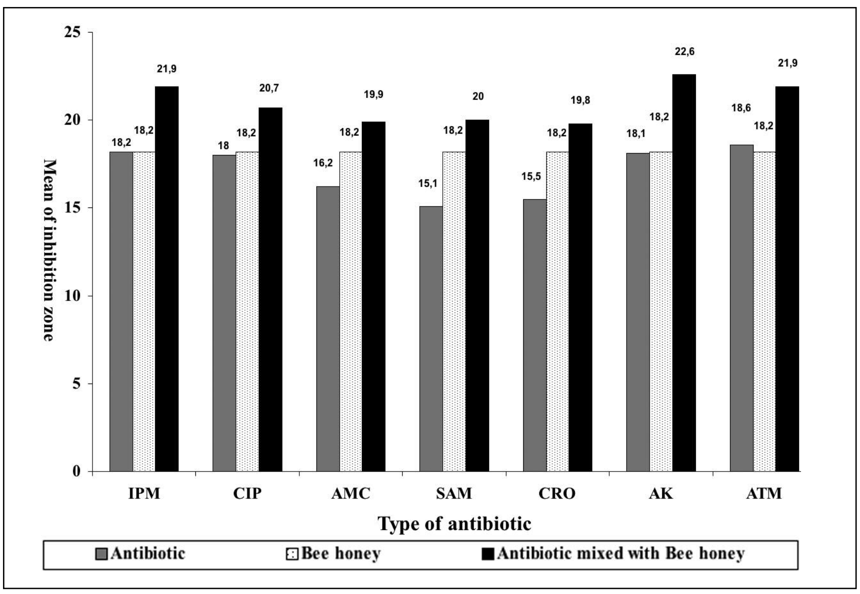

Fig. 2 - Inhibitory zones in cm of antibiotics, bee honey, and a combination of both on 21 isolated gram-negative bacilli: comparative study.

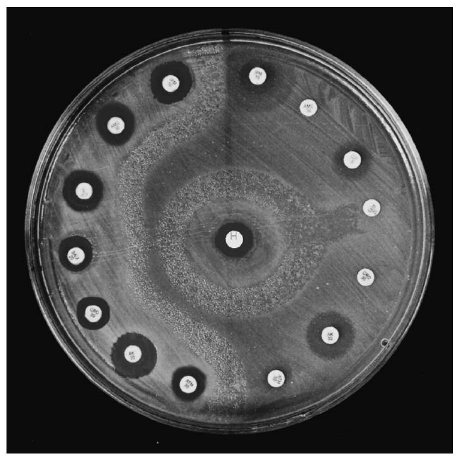

Fig. 3 - Sensitivity of *Pseudomonas aeruginosa* to antibiotics, bee honey, and antibiotics mixed with honey: comparative study.

bacilli were sensitive to IPM (85.7%), CIP (28.6%), AMC (28.6%), SAM (38.1%), CRO (38.1%), AK (100%), and ATM (57.1%), while 85.7% of the isolated bacilli were sensitive to honey.

The mean inhibition zone of honey (18.2 ± 2.5 mm) was significantly higher than that of antibiotics: AMC, SAM, and CRO ($p_1 = 0.005$ for each) and insignificantly higher than AK and CIP alone ($p_1 > 0.05$ for each). The mean inhibition zone of honey was however non-significantly lower than that of ATM (18.6 ± 3.1 mm) ($p_1 > 0.05$) and was more or less similar to that of IPM (18.2 ± 4.2 mm).

When honey was added to the antibiotic discs, there was a significant increase in the mean inhibition with respect to honey for IPM, AK, and ATM ($p_2 < 0.001$ for each) and for CIP, AMC, SAM, and CRO ($p_2 \leq 0.05$). There were significant increased inhibition zones of antibiotics mixed with honey compared with inhibition zones of antibiotics alone: CIP, AMC, SAM, CRO, and AK ($p_3 < 0.001$ for each), while the increase was significant for IPM and ATM ($p_3 = 0.005$ for each).

Table II and *Figs. 4* and *5* show the effect of antibiotics, honey and a combination of both on isolated coagulase-positive staphylococci. All the coagulase-positive staphylococci isolated (100%) were methicillin-resistant (methicillin-resistant *Staphylococcus aureus* [MRSA]). They were sensitive to IPM (88.9%), VA (77.8%), CIP (55.6%), SAM (55.6%), AMC (44.4%), and CRO (44.4%),

Table II - Effect of antibiotics, bee honey, and a combination of both on isolated coagulase-positive staphylococci (no. = 9)

	Antibiotic		Bee honey		Antibiotic + bee honey		*p*		
	No. (%)	Inhibition zone in cm (mean ± S.D)	No. (%)	Inhibition zone in cm (mean ± S.D)	No. (%)	Inhibition zone in cm (mean ± S.D)	p_1	p_2	p_3
IPM	8 (88.9%)	17.9 ± 3.5	9 (100%)	18.7 ± 2.2	9 (100%)	21.8 ± 4.4	>0.05	0.025	0.025
CIP	5 (55.6%)	16.8 ± 1.3			9 (100%)	21.2 ± 2.3	0.025	0.01	<0.001
AMC	4 (44.4%)	20.8 ± 0.9			9 (100%)	22.6 ± 2.2	0.025	0.001	0.025
SAM	5 (55.6%)	16.4 ± 2.6			9 (100%)	21.0 ± 2.6	0.05	0.025	0.005
CRO	4 (44.4%)	16.3 ± 1.5			9 (100%)	21.4 ± 3.3	0.025	0.025	0.005
ME	0 (0%)	0			9 (100%)	20.6 ± 2.4	<0.001	0.05	<0.001
VA	7 (77.8%)	15.6 ± 3.8			9 (100%)	21.3 ± 2.6	0.05	0.01	<0.001

p_1 = bee honey versus antibiotic
p_2 = antibiotic mixed with bee honey versus bee honey alone
p_3 = antibiotic mixed with bee honey versus antibiotic alone
p is significant if ≤ 0.05

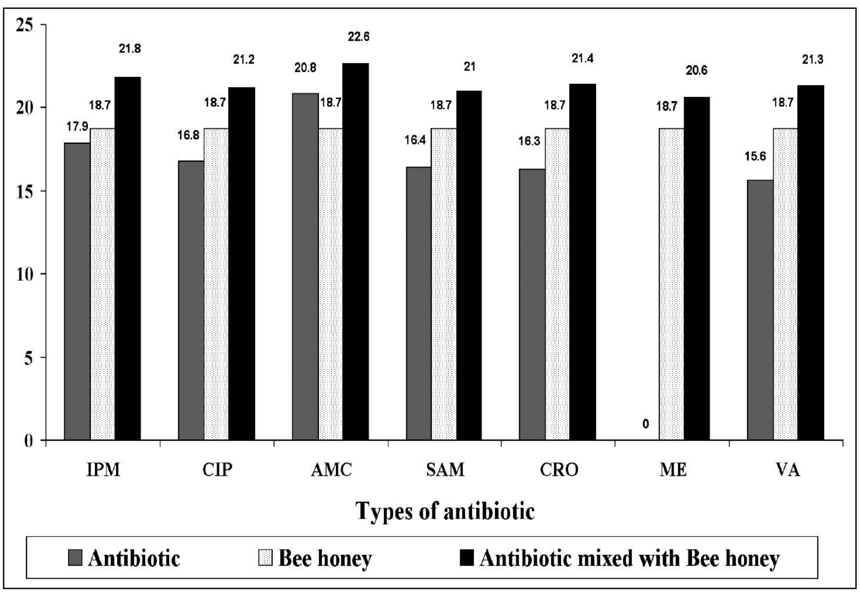

Fig. 4 - Inhibitory zones of antibiotics, bee honey, and a combination of both on nine isolated staphylococci: comparative study.

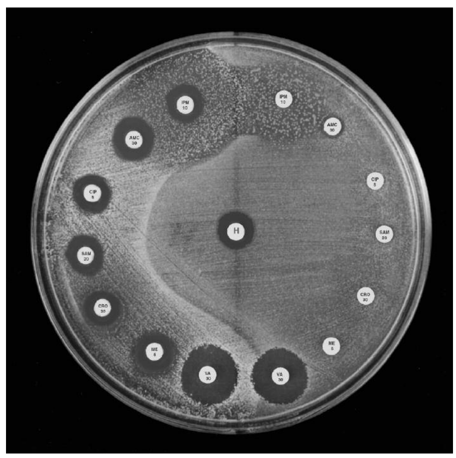

Fig. 5 - Sensitivity of staphylococci to antibiotics, bee honey and antibiotics mixed with honey: comparative study.

while all the staphylococci isolated (100%) were sensitive to honey.

The mean inhibition zone of honey (18.7 ± 2.2 mm) was significantly higher than that of antibiotics (CIP, SAM, CRO, and VA ($p_1 \leq 0.05$) and ME ($p_1 < 0.001$), while there was a non-significant increase in the mean inhibitory zone produced by honey compared with that of IPM ($p_1 > 0.05$). However, the mean inhibition zone of honey was significantly lower than that of AMC (20.8 ± 0.9 mm) ($p_1 = 0.025$).

After addition of honey to the antibiotic discs, there was a significant increase in the mean inhibition zones that was greater than for honey alone in IPM, CIP, AMC, SAM, CRO, ME, and VA ($p_2 \leq 0.05$).

There were highly significant increased inhibition zones of antibiotics mixed with honey with respect to honey in CIP, VA, and ME ($p_3 < 0.001$ for each) and in IPM, AMC, SAM, and CRO ($p_3 \leq 0.05$).

Discussion

Despite recent advances in antimicrobial chemotherapy and burn wound management, infection continues to be an important problem in the treatment of burns. A variety of topical agents such as silver sulphadiazine, silver nitrate, and sulphamylon, as well as systemic agents such as penicillins, monobactams, cephalosporins and aminoglycosides, also have been used, but none has completely eliminated the problem of infection.[3]

Honey is the most famous rediscovered remedy that has been used to promote wound and burn healing and also to treat infected wounds.[4]

The antimicrobial activity of bee honey has been attributed to several properties of honey, including its osmotic effect, its naturally low pH, and the production of hydrogen peroxide, as also the presence of phenolic acids, lysozyme, and flavanoids.[5]

The aim of the present study is to evaluate the antimicrobial effect of bee honey on organisms isolated from infected burns compared with that of antibiotics used in burn infection treatment and to evaluate the effects produced when bee honey was added to antibiotic discs.

In this study, *Pseudomonas aeruginosa* was the organism most frequently isolated, representing 16 (53.3%) of the isolates, followed by coagulase-positive staphylococci (9, i.e. 30%) and then *Enterobacter* spp. (3, i.e. 10%). *Klebsiella*, with two isolates (6.7%), was the least frequently isolated organism.

Douglas et al.[6] stated that *Pseudomonas aeruginosa* continued to be a serious cause of infection and septic mortality in burn patients, particularly when nosocomially acquired, and that all recent efforts were directed to solve this problem. Agnihotri et al.[7] reported that *Pseudomonas aeruginosa* was the commonest isolate (59%), followed by *Staphylococcus aureus* (17.9%), *Acinetobacter* spp. (7.2%), *Enterobacter* spp. (3.9%), *Klebsiella* spp. (3.9%), *Proteus* spp. (3.3%), and others (4.8%).

The mean inhibition zone (in mm) of honey, when applied on isolated gram-negative bacteria (*Pseudomonas aeruginosa*, *Enterobacter*, and *Klebsiella*), was found to be 18.2 ± 2.5 mm, which was significantly higher than that of AMC, SAM, and CRO ($p_1 = 0.005$ for each) and non-significantly higher than that of AK and CIP ($p_1 > 0.05$ for each). The mean inhibition zone of honey was however non-significantly lower than that of ATM ($p_1 > 0.05$), while it was more or less similar to that of IPM (18.2 ± 4.2 mm).

The effect of honey on gram-negative bacteria was explained by Taormina et al.,[8] who attributed it to the presence in bee honey of hydrogen peroxide and powerful antioxidants, as also to a naturally low pH, which is unsuitable for bacterial growth, and to the presence of phenolic acids, lysozyme, and flavanoids.

When honey was added to the antibiotic discs, there was a highly synergistic increase in the mean inhibition zones in IPM, AK, and ATM compared with honey ($p_2 < 0.001$ for each), while there was a significant increase in CIP, AMC, SAM, and CRO ($p_2 < 0.05$ for each).

In a similar study, Karayil et al.[9] observed an *in vitro* synergistic effect of honey when added to antibiotics, especially aminoglycosides, on multidrug-resistant organisms, and in particular on *Pseudomonas aeruginosa*, which is the commonest organism that causes burn wound infection.

There were highly significant increased inhibition zones with regard to antibiotics mixed with honey compared with inhibition zones of antibiotics alone, i.e. CIP, AMC, SAM, CRO, and AK ($p_3 < 0.001$ for each), while the increase was significant in IPM and ATM ($p_3 = 0.005$ for each).

The above observations indicate that honey had a more inhibitory effect on isolated gram-negative bacilli than amoxicillin/clavulinic acid, sulbactam/ampicillin, and ceftriaxone and a more or less similar effect to that of ciprofloxacin, imipenem, amikacin, and aztreonam. With the addition of honey to the antibiotics, there was a synergistic effect in all cases (*Table I*).

Fish et al.[10] documented that imipenem is frequently used in critically ill patients owing to its broad spectrum of potent antimicrobial activity against many common gram-positive and gram-negative pathogens, including *Pseudomonas aeruginosa*.

Resistance to the commonly used systemic antibiotics, especially betalactams and aminoglycosides, is a major problem - the effectiveness of multiple antibiotics has recently been observed to be decreasing.[11]

As the understanding of the mechanisms of resistance and the rapidity of development of *Pseudomonas* has increased, it has generally become accepted that serious *Pseudomonas* infections should be treated with two antibiotics that act with two different action mechanisms that are synergistic in their activity.[12] Our study showed a synergistic effect for honey when added to antibiotics.

Gram-positive bacteria, including *Staphylococcus aureus*, affect burn wounds during the first week following a trauma. Colonization with *Staphylococcus aureus* is often associated with delayed wound healing, an increase in the need for surgical interventions, and prolonged stay in the burns centre. The transmission of *Staphylococcus aureus* is a frequent occurrence, involving both patients and persons in close contact with them.[13]

Our *in vitro* study (*Table II*) of the susceptibility of isolated staphylococci revealed the synergistic effect of honey added to the tested antibiotic discs. The mean inhibition zone of honey (18.7 ± 2.2 mm) on MRSA was significantly higher than that of CIP, SAM, CRO, and VA ($p_1 < 0.05$) and of ME ($p_1 < 0.001$), while there was a non-significant increase in the inhibitory zone produced by honey compared with that of IPM ($p_1 > 0.05$). The mean inhibition zone of honey was however significantly lower than that of AMC (20.8 ± 0.9 mm) ($p_1 = 0.025$).

Reporting on clavulanate, a broad-spectrum ß-lactamase inhibitor with activity against both gram-positive and gram-negative bacteria, Finlay et al.[14] found that clavulanate alone had minimal antibacterial activity, while a combination of clavulanate with amoxicillin or ticarcillin had been widely and effectively used in the treatment of a broad range of clinical infections.

In the present study, after the addition of honey to the antibiotic discs, there was a significant increase in the mean MRSA inhibition zones with regard to IPM, CIP, AMC, SAM, CRO, ME, and VA) with regard to both honey ($p_2 < 0.05$) and antibiotics alone ($p_3 < 0.05$).

It was observed that honey was more effective than ciprofloxacin, sulbactam/ampicillin, ceftriaxone, methicillin, and vancomycin but less effective than amoxicillin/clavulinic acid. On the addition of honey to the antibiotic discs, there was a synergistic effect with all antibiotics.

Honey showed itself to be a cheap, non-toxic remedy with significant antimicrobial effect on gram-positive bacteria isolated from burn wounds, especially MRSA, which is considered the commonest gram-positive coccus affecting such wounds. In addition, honey does not affect human tissue adversely, unlike some other topical antimicrobial agents.[15]

Conclusions

Honey showed a greater inhibitory effect (85.7%) on isolated gram-negative bacteria (*Pseudomonas aeruginosa, Enterobacter* spp., and *Klebsiella*) than the antibiotics commonly used (imipenem, ciprofloxacin, amoxicillin/clavulinic acid, sulbactam/ampicillin, ceftriaxone, and aztreonam).

Honey showed an inhibitory effect on all (100%) MRSA, compared with all commonly used antibiotics (imipenem, ciprofloxacin, amoxicillin/clavulinic acid, sulbactam/ampicillin, ceftriaxone, vancomycin, and methicillin).

When added to antibiotics, honey had a synergistic effect on gram-negative bacteria and also on coagulase-positive staphylococci.

RÉSUMÉ. Malgré les progrès récents dans le secteur de la chémothérapie antimicrobienne et la gestion des lésions causées par les brûlures, l'infection reste un important problème pour ce qui concerne les brûlures. Le miel est le plus fameux des remèdes redécouverts utilisés pour traiter les lésions infectées et favoriser la guérison. Les Auteurs de cette étude évaluent l'effet antimicrobien du miel des abeilles sur les organismes isolés dans les lésions infectées en comparaison avec les antibiotiques utilisés dans le traitement des infections dues aux brûlures et en outre ils considèrent les effets obtenus quand on ajoute du miel aux disques antibiotiques. Trente patients atteints d'infection due à une brûlure ont été sélectionnés pour l'étude. Les spécimens collectionnés ont été cultivés sur des plats d'agar de sang. Les colonies isolées ont été identifiées avec diverses modalités. Les organismes isolés ont été inoculés sur agar Müller-Hinton. Chaque plat a été divisé avec un feutre permanent en deux parts égales. Les disques antibiotiques ont été étalés dans une part et, dans l'autre, chaque disque antibiotique, immergé en miel, a été étalé du côté contraire du même disque antibiotique. Au centre de l'agar, un disque de papier filtre stérile immergé en miel a été appliqué. L'organisme isolé le plus fréquemment était le *Pseudomonas aeruginosa*, qui représentait le 53,3% des isolés. Les zones d'inhibition moyenne (exprimées en mm) produites par le miel (18,2 ± 2,5 mm) appliqué aux bactéries à gram négatif isolées étaient significativement plus élevées par rapport à celles de l'amoxicilline/acide clavulinique, le sulbactam/ampicilline et la ceftriaxone ($p_1 = 0.005$ en chacun). Quand le miel a été ajouté aux disques antibiotiques, la sensibilité des bactéries à gram négatif isolées augmentait en manière hautement significative par rapport à chacun des disques à seul et au miel seul. La susceptibilité des staphylocoques isolés révélait l'effet synergistique du miel ajouté aux disques antibiotiques testés. L'effet antimicrobien du miel (18,7 ± 2,2 mm) était significativement plus élevé par rapport aux antibiotiques, c'est-à-dire la ciprofloxacine, le sulbactam/ampicilline, la ceftriaxone et la vancomycine ($p_1 \leq 0.05$ en chacun). Après l'addition du miel aux disques antibiotiques testés, les zones d'inhibition des antibiotiques ajoutés au miel augmentaient en manière hautement significative par rapport aux seuls antibiotiques, c'est-à-dire ciprofloxacine, vancomycine et méthicilline ($p_3 \leq 0.001$ en chacun). En outre, l'incrément était significatif par rapport aux antibiotiques seuls, c'est-à-dire l'imipénem, l'amoxicilline/acide clavulinique et la ceftriaxone ($p_3 \leq 0.05$). Pour conclure, le miel a démontré un effet inhibitoire majeur (85,7%) sur les bactéries à gram négatif isolées (*Pseudomonas aeruginosa, Enterobacter* spp., *Klebsiella*) par rapport aux antibiotiques communément utilisés, tandis qu'il avait un effet inhibitoire sur tous les *Staphylococcus aureus* résistants à la méthicilline (100%) par rapport aux seuls antibiotiques. Un effet synergistique du miel a été observé quand il a été ajouté aux antibiotiques pour les bactéries à gram négatif et aussi pour les staphylocoques à coagulase-positive.

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